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Mediated amperometric monitoring of DT-diaphorase induction in cancer cells – tool for screening phytotherapeutical drugs

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Chemotherapy has been used as a treatment of cancer during the past 60 years. However, chemotherapeutic drugs are well known for undesirable side effects due to unspecificity of the drugs and genetic variation between patients. One approach to eliminate unspecificity is *targeted chemotherapy* [1] using prodrugs, which are selectively activated in tumor tissue without affecting healthy tissues. Activation of many prodrugs is achieved through intracellular reduction by cytosolic redox enzymes (*bioreductive activation*), the most important of which is DT-diaphorase. In many type of cancer cells, DT-diaphorase is naturally overexpressed, whereas it is not the case in healthy tissues [2]. There are, however, cancer cells that do not possess a significant DT-diaphorase activity. Intensive research is ongoing to identify synthetic or naturally occurring chemical species that can selectively induce DT-diaphorase activity in cancer cells to widen the applicability of chemotherapeutic drugs relying on bioreduction [3]. Traditional screening assays based on enzyme activity determination in cell lysates are very labour-intensive and time consuming.

Here, we present optimization of a mediated amperometric assay for monitoring induction of DT-diaphorase activity in living cancer cells as the first demonstration of the technique on living human cells. Detection is conducted based on the application of the double mediator system menadione/ferricyanide (Fig. 1). In our previous studies, these mediators have been applied successfully for screening of genetic modifications of *S. cerevisiae* cells in relation to cellular redox activity [4,5]. Menadione, a lipophilic quinone capable of entering the intracellular environment, is reduced by cytosolic redox enzymes, e.g., DT-diaphorase. Upon diffusing back to the extracellular environment, the reduced form of menadione delivers the electrons to ferricyanide, which is reoxidized at an electrode. The recorded current is an indication of changes in cellular reducing capacity. The project aims at assay automation using a modular microfluidic cell culture device analogously with our previous studies on *S. cerevisiae* [6,7].

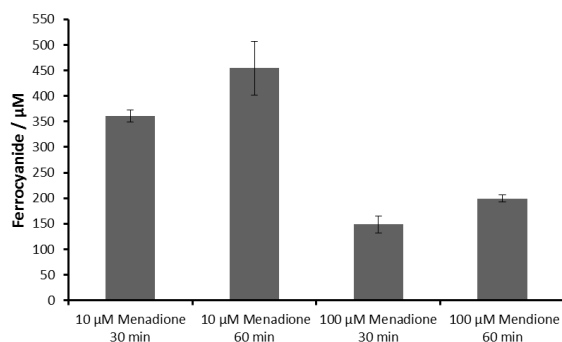


Figure 1. The effect of menadione concentration and assay time on cellular reducing capacity in HeLa cells

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